Serial No.: 09/770,770
Filing Date: January 25, 2001

REMARKS

Claims 1-16 are pending in the instant application. A version of the claims indicating the amendments being made in this response is attached under the heading "Version Showing Changes Made." A version of the claims after entry of these amendments is attached under the heading "Appendix of Pending Claims."

The Invention

The instant invention relates to methods for screening for and identifying sequence alterations in nucleic acids. In these methods a number of nucleic acid probes are hybridized to completely complementary regions of a control nucleic acid as well as to a target nucleic acid that may or may not be completely complementary. By measuring the dissociation temperatures of the various probes with either the control or the target nucleic acid, it is possible to determine if differences are present between the control and target nucleic acids. Furthermore, by using overlapping probes in these methods it is possible to use differences in dissociation temperature to indicate the exact location and identity of any differences.

In one method, a plurality of overlapping probes are used to identify sequence alterations. These probes, when taken together, are an exact complement to a control nucleic acid sequence. The probes are individually hybridized to a target sequence and the dissociation temperature, T_m, is measured for each individual probe bound to the target. These T_ms are then compared to the T_ms of the individual probes when bound to the control sequence. The difference between the two T_ms for any one probe is termed the ΔT_m. A change in the dissociation temperature between the probe/control pair and the probe/target pair is indicative of a difference in sequence between the control and the target sequences. Because of the overlapping nature of the probes, there will always be a second probe that hybridizes at the site of the difference in sequence between control and the target sequences. By comparing the ΔT_m of the first probe and the ΔT_m of the second probe you are able to determine the difference in ΔT_m ($\Delta\Delta T_m$). Because every sequence

alteration will cause a distinctive change in the ΔT_m, the $\Delta\Delta T_m$ can be used to indicate the location and identity of the nucleotide difference between the control and target sequences.

In another method, a plurality of nucleic acid probes, again complementary to regions of a control sequence, are hybridized to a target sequence and T_ms are measured. The difference between this method and the one described above is that a first set of nucleic acid probes complementary to regions of the control sequence separated by one or more nucleotides are used along with at least a second set of nucleic acid probes complementary to regions of the control sequence that are also separated by one or more nucleotides. Furthermore, the regions complementary to the secondary set (or sets) of probes include the nucleic acids separating the first set of probes as well as overlapping with the regions complementary to the first set of probes. By determining the ΔT_ms and $\Delta\Delta T_m$s, as described above, one can find the location and identity of alterations between the control and target sequences.

Step c) of Claims 1 and 6

The Examiner asserts that step c) of Claims 1 and 6 are not clear in explaining how ΔT_m is determined.

Step c) of Claims 1 and 6 has been amended to make clear that ΔT_m is determined, as discussed above, by calculating the difference in melting temperature of an individual probe from the control sequence and that same probe's melting temperature from a target sequence. Accordingly, each probe will have its own ΔT_m.

Step d) of Claims 1 and 6

The Examiner asserts that step d) of Claims 1 and 6 are not clear in explaining how the difference in ΔT_m is determined.

Step d) of Claims 1 and 6 has been amended to make clear that the difference in ΔT_m is determined, as discussed above, by calculating the difference, if any, between the ΔT_m of one probe and the ΔT_m of an overlapping probe.

CONCLUSION

Applicants respectfully submit that the above proposed amendments and remarks would overcome all of the concerns raised by the Examiner and therefore all claims would be in condition for allowance. If, upon review, the Examiner feels there are additional outstanding issues, the Examiner is invited to call the undersigned attorney. This paper is filed under 37 C.F.R. section 1.34(a).

Respectfully submitted,
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Date: _____

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VERSION TO SHOW CHANGES MADE

1. A method of identifying a sequence alteration in a target nucleic acid as compared to a control nucleic acid, said method comprising:

a) hybridizing a plurality of nucleic acid probes with said target nucleic acid, wherein said probes are complementary to different overlapping regions of said control nucleic acid;

b) determining the melting temperature (T_m) of at least two overlapping probes from said target nucleic acid;

c) determining the ΔT_m for each of said overlapping probes, wherein the ΔT_m is [for said at least two probes] the difference between the T_m of [from] said target nucleic acid and one of said overlapping probes and the T_m of [from] said control nucleic acid and the same overlapping probe [(ΔT_m)]; and

d) determining the difference, if any, in [determined] ΔT_m [between] of at least two overlapping probes as an indication of the presence or absence of a sequence alteration in said target nucleic acid as compared to said control nucleic acid, wherein the differences in ΔT_m indicate a sequence alteration in said target nucleic acid.

6. (Amended) A method of identifying a sequence alteration in a target nucleic acid as compared to a control nucleic acid, said method comprising:

a) hybridizing a plurality of nucleic acid probes with said target nucleic acid, wherein a first set of probes is complementary to regions of said control nucleic acid separated by one or more nucleotides and at least a second set of probes is complementary to regions of said control separated by one or more nucleotides, wherein the regions complementary to said second set of probes include the nucleic acids separating the first set of probes and are overlapping with the regions complementary to said first set of probes;

b) determining the melting temperature (T_m) of at least two overlapping probes from said target nucleic acid;

c) determining the ΔT_m for each of said overlapping probes, wherein the ΔT_m is [for said at least two probes] the difference between the T_m of [from] said target nucleic acid and one of said overlapping probes and the T_m of [from] said control nucleic acid and the same overlapping probe [(ΔT_m)]; and

d) determining the difference, if any, in [determined] ΔT_m [between] of at least two overlapping probes ~~as an indication of the presence or absence of a sequence alteration in said target nucleic acid~~ as compared to said control nucleic acid.